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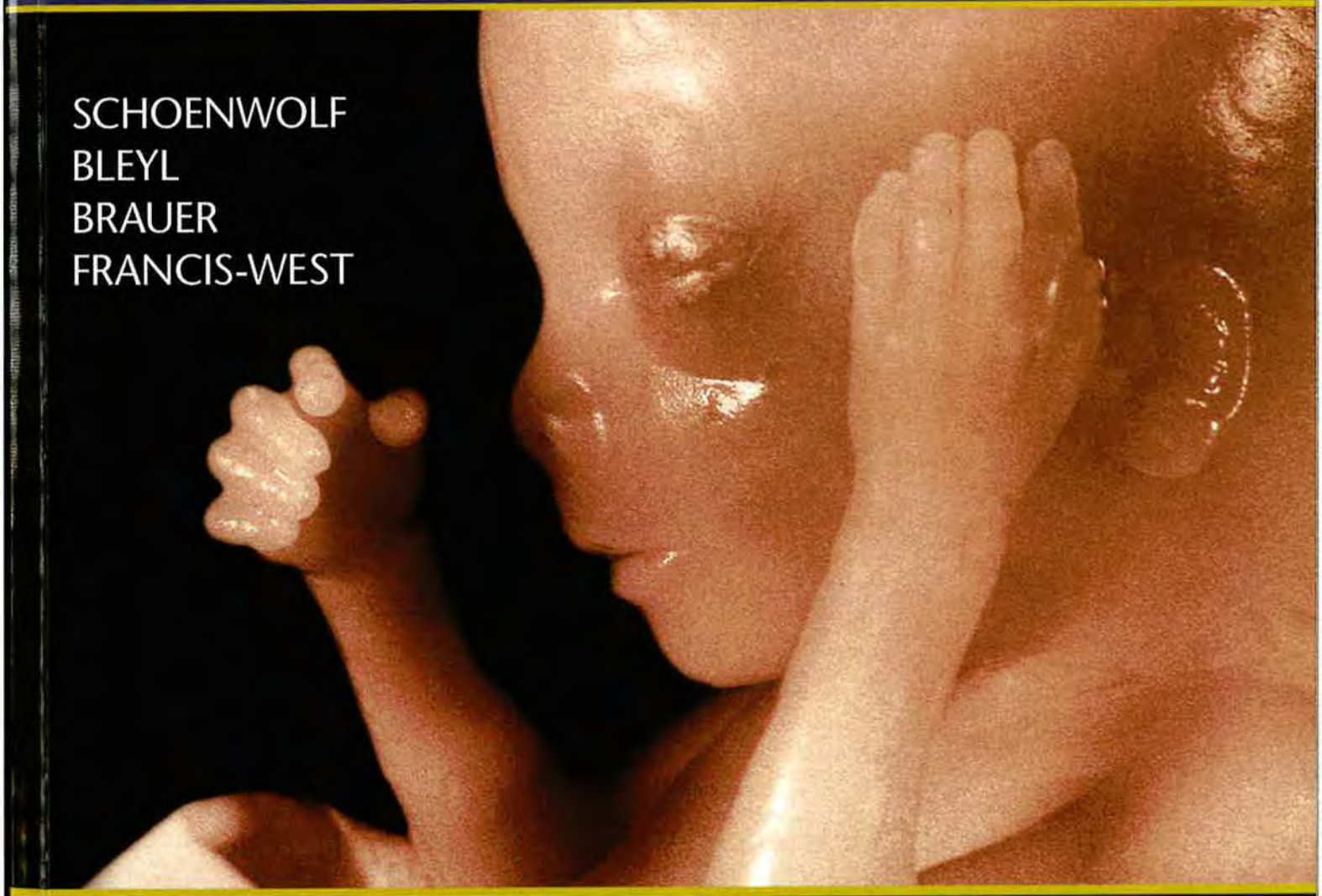

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FIFTH EDITION

Larsen's Human Embryology

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Chapter 12
Development of the Heart

SUMMARY

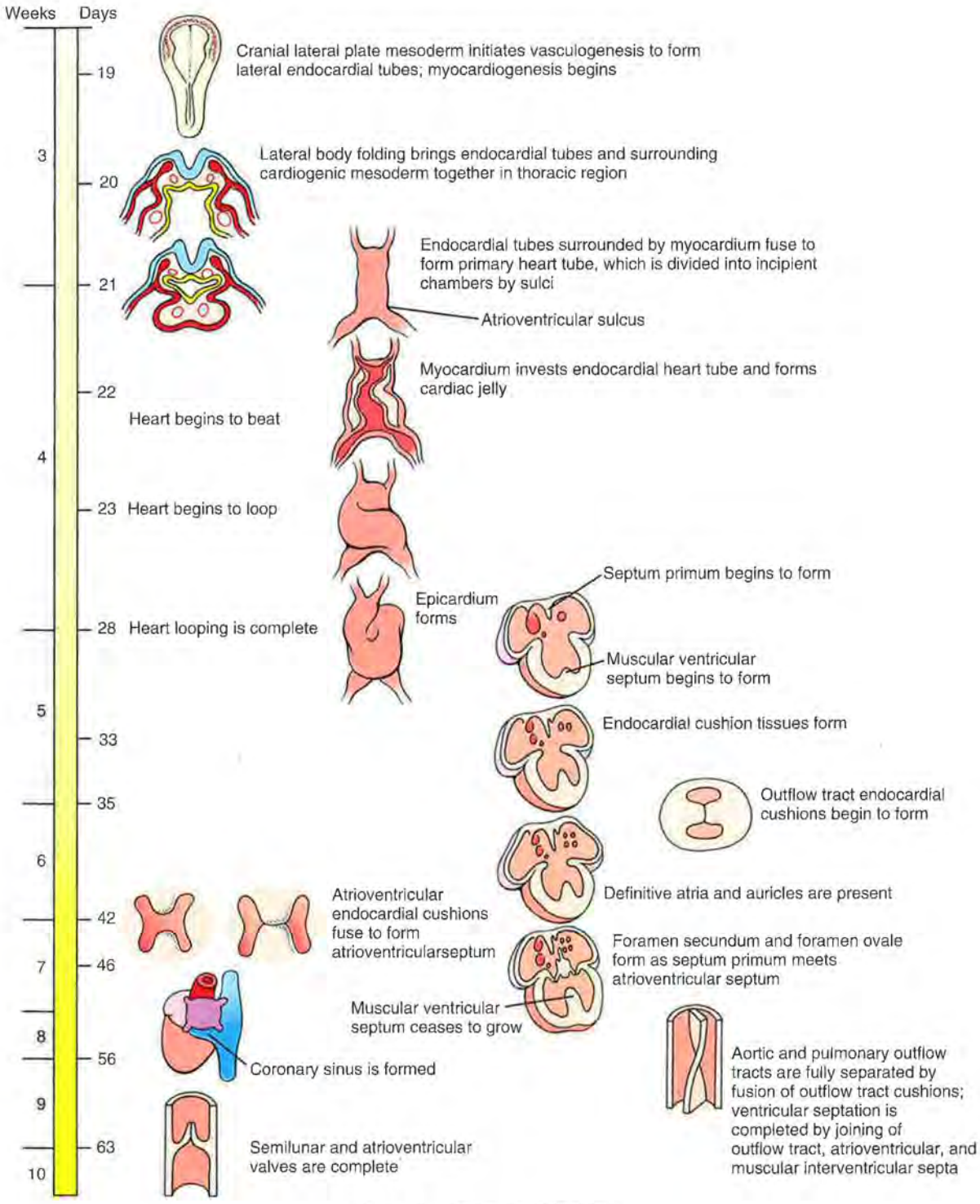
In response to inductive and permissive signals emanating from the endoderm, ectoderm, and midline mesoderm, cardiogenic precursors form a cardiac primordium within the splanchnic mesoderm at the cranial end of the embryonic disc called the **cardiac crescent**, or **first heart field**. In response to signals from the underlying endoderm, a subpopulation of cells within the first heart field form a pair of **lateral endocardial tubes** through the process of **vasculogenesis**. The cranial and lateral folding of the embryo during the fourth week results in the fusion of these tubes along the midline in the future thoracic region, where they form a single **primary heart tube**. This tube consists of a single endocardial tube with adjacent mesoderm differentiating into cardiomyocytes.

The **heartbeat** is initiated around the twenty-first day, and its continual beating is required for normal heart development. Between weeks four and eight, the primary heart tube undergoes a series of events, including **looping**, **remodeling**, **realignment**, and **septation**, eventually leading to the transformation of a single heart tube into a four-chambered heart, thus laying down the basis for the separation of pulmonary and systemic circulations at birth.

Starting at the inflow end, the primary heart tube initially consists of the **left** and **right horns of the sinus venosus**, the **primitive atrium**, the **atrioventricular canal**, the **primitive left ventricle**, and a short outflow region. Lengthening of the primary heart tube and proper cardiac bending and looping are driven through the addition of cardiac precursor cells by the **second heart field**. At the outflow end, the main additions are the **primitive right ventricle** and the **outflow tract** that connects with the **aortic sac** at the arterial orifice. As the outflow tract lengthens, proximal (conus) and distal (truncus) components can be distinguished. Septation of the outflow tract leads to separate left and right ventricular outlets and to formation of the ascending aorta and pulmonary trunk. At the inflow end, the second heart field also contributes myocardium to the sinus venosus wall, the body of the **right** and **left atrium**, and the **atrial septa**.

Venous blood initially enters the sinus horns through paired, symmetrical **common cardinal veins**. However, as covered in Chapter 13, changes in the venous system rapidly shift the entire systemic venous return to the right, so that all blood from the body and umbilicus enters the future right atrium through the developing **superior** and **inferior caval veins**. The left sinus horn becomes the **coronary sinus**, which collects blood from the coronary circulation. A process of intussusception incorporates the right sinus horn and the ostia of the **caval veins** into the posterior wall of the future right atrium. In this process, the **pulmonary vein** developing within the dorsal mesocardium shifts to the future left atrium as a result of the development of a **dorsal mesenchymal protrusion**. Subsequently, the walls of the pulmonary vein are partially incorporated into the atrial wall, forming the larger part of the dorsal left atrial wall. In the fifth and sixth weeks, the atrial septum starts to develop. This is a two-step process. It begins with the formation of the **septum primum (primary atrial septum)**, which is followed by formation of the **septum secundum (secondary atrial septum)**. The formation of this atrial septal complex results in separation of the right and left atria. However, the two septa do not fuse until after birth, allowing for right-to-left shunting of blood throughout gestation. The **mitral (bicuspid)** and **tricuspid atrioventricular valves** develop from atrioventricular cushion tissue during the fifth and sixth weeks. Meanwhile, the heart undergoes remodeling, bringing the future atria and ventricles into correct alignment with each other and aligning both ventricles with their respective future outflow vessels. During expansion of the primitive right and left ventricles, a **muscular ventricular septum** forms that partially separates the ventricles. During the seventh and eighth weeks, the outflow tract of the heart completes the process of septation and division. During this process, remodeling of the distal outflow tract cushion tissue (truncal cushions) results in the formation of the **semilunar valves** of the aorta and pulmonary artery. Fusion of the proximal outflow tract cushions (conal cushions) creates the outlet septum, resulting in the separation of left and right ventricular outlets. Complete ventricular septation depends on fusion of the outflow tract (conotruncal) septum, the muscular ventricular septum, and the atrioventricular cushion tissues.

The myocardium of the heart differentiates into working myocardium and myocardium of the **conduction system**. The **epicardium** grows out from the **proepicardial organ** covering the myocardium. It contributes to the formation of the coronary vasculature, which is necessary for oxygenation of the thickening myocardial wall and myocardial cell population.



Time line. Formation of the heart.

Clinical Taster

A full-term boy is born to a primigravid (first gestation) mother after an uncomplicated pregnancy. The delivery goes smoothly, with healthy Apgar scores of 8/10 at one minute and 9/10 at five minutes. All growth parameters (length, weight, and head circumference) are normal, ranging between the 10th and 25th centiles. The newborn examination is also normal, and the infant is returned to his mother to begin breast feeding.

The boy initially feeds well, but he becomes sleepy and disinterested in feeding as the day progresses. At twenty hours after birth, he exhibits decreased peripheral perfusion, cyanosis, and lethargy. A pulse oximeter shows oxygen saturation in the low 80% range (normal equals >90%) with increasing **respiratory distress**. Paradoxically, blood oxygen saturation worsens after administration of oxygen. The boy is emergently transferred to the neonatal intensive care unit in worsening shock. There, he is intubated, central intravascular catheters are placed, and he is started on **prostaglandins**.

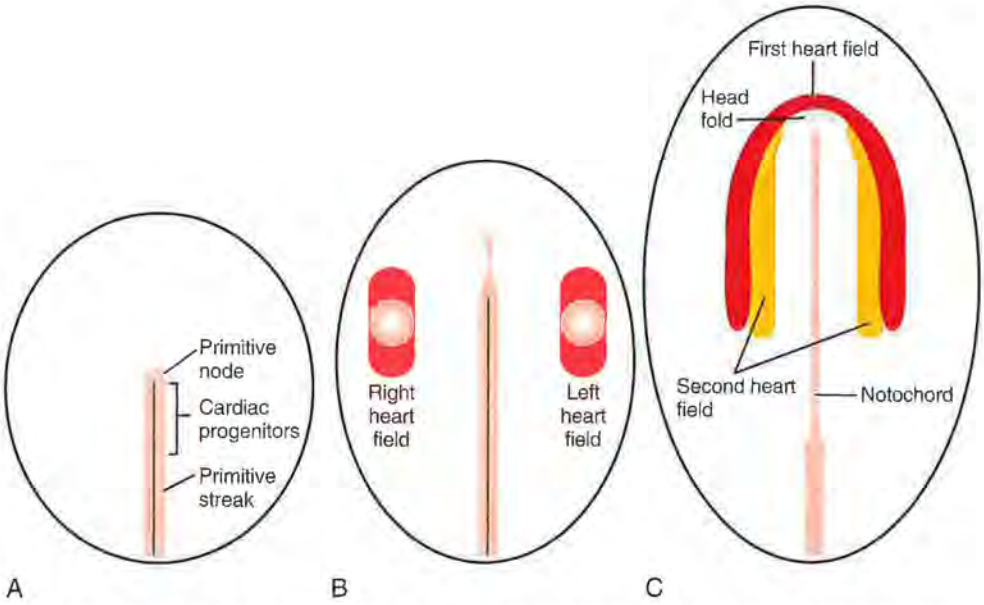


Figure 12-1. Formation of the first heart field seen in ventral views. *A*, Location of cardiogenic progenitors in the early primitive streak. *B*, Location of cardiogenic precursors (red gradated regions) within the mesoderm shortly after gastrulation and during initial specification. *C*, Location of the first heart field (red) containing specified cardiogenic cells. The crescent-like arrangement of the progenitors is due to their migration pattern, local cardiogenic induction signals, and development of the body folds. Medial and slightly caudal to the first heart field lies the second heart field (orange).

A chest x-ray shows **cardiomegaly** (enlarged heart) and increased pulmonary vascularity (indicative of increased blood flow). An **echocardiogram** shows a very small left ventricle with a small aortic outflow tract, leading to the diagnosis of **hypoplastic left heart syndrome** (HLHS).

HLHS is a shunt-dependent lesion: survival of these patients depends on maintaining a **patent ductus arteriosus** (PDA) to carry blood from the pulmonary artery to the aorta and out to perfuse the systemic circulation. Supplemental oxygen lowers resistance to pulmonary blood flow, causing blood to circulate to the lungs instead of crossing the PDA. Thus, administering supplemental oxygen actually decreases blood oxygen saturation. Administration of prostaglandins prevents the physiological closure of the ductus arteriosus, maintaining systemic perfusion until surgery can be performed. The first-stage surgery, called the Norwood procedure, connects the right ventricular outflow tract to the aorta, and a separate shunt is used to provide blood flow to the lungs. More surgeries follow at about six months and two to three years of age. Occasionally, heart transplantation is performed. The five-year survival rate for HLHS is around 70%.

ESTABLISHING CARDIAC LINEAGE

The heart is the first organ to function in human embryos. It begins beating as early as the twenty-first day, and starts pumping blood by the twenty-fourth to twenty-fifth day. Much of cardiac development, including remodeling and septation, occurs while the heart is pumping blood. This is necessary to provide nutrients and oxygen and to dispose of wastes during embryonic and fetal development, but this mechanical and electrical activity also plays an important role in the morphogenesis of the heart. The embryonic heart is first morphologically identifiable as a single tube composed of contractile myocardium surrounding an inner endocardial (endothelial) tube, with an intervening extracellular matrix. The heart is also an asymmetrical organ whose left-right patterning is established during gastrulation (left-right patterning is covered in Chapter 3 and later in this chapter).

Cardiac progenitor cells are derived from intraembryonic mesoderm emerging from the cranial third of the primitive streak during early gastrulation. These progenitors leave the primitive streak and migrate in a cranial-lateral direction to become localized on either side of the primitive streak (Fig. 12-1*A, B*). The cardiac progenitor cells eventually become localized within the cranial lateral plate mesoderm on both sides of the embryo, extending and arcing cranial to the developing head fold, forming a **cardiac crescent** (Fig. 12-1*C*). Cells in the cardiac crescent constitute the so-called **first heart field**. It is thought that the cardiac cell lineage is specified from mesodermal cells within the first heart field. As discussed later, the first heart field is not the sole source of cardiogenic cells for the developing heart, as medial to the first heart field, there is already a population of **second heart field** cells (Fig 12-1*C*).

In the Research Lab

SPECIFICATION OF CARDIAC PROGENITOR CELLS

To what degree cardiac progenitor cells within the epiblast and the primitive streak are specified remains unknown. Activin and Tgfβ produced by the hypoblast of the chick induce cardiogenic properties in some of the overlying epiblast cells (Fig. 12-2*A, B*). Other members of the Tgfβ superfamily, including nodal and Vg1, also play a role in inducing cardiogenic properties in the epiblast. During gastrulation, cardiac precursors residing in the primitive streak are uncommitted, but these progenitors become specified to become cardiogenic mesoderm soon after migrating into the lateral plate. Mesp1 (mesoderm posterior 1) and Mesp2 (mesoderm posterior 2), members of the basic HLH family of transcription factors, are expressed transiently during the primitive streak stage. Both are required for migration of the cardiac progenitor cells into the cranial region of the embryo, and both have been implicated in the specification of the early cardiovascular

lineage. Interaction of cranial lateral mesoderm with the endoderm is required for this cardiac specification. The endoderm secretes several signaling molecules—including Bmp, Fgf, activin, insulin-like growth factor 2, and Shh—that promote cell survival and proliferation of cardiogenic cells. One particularly important growth factor is Bmp2, which is essential for stimulating the expression of early cardiogenic transcription factors, such as Nkx2.5 (Nkx2 transcription factor related, locus 5) and Gata (proteins that bind to a DNA GATA sequence) within the lateral mesoderm. In the chick embryo, Bmp2 can induce expression of myocardial cell markers in ectopic regions (i.e., outside their proper position), whereas mouse embryos lacking Bmp2 fail to develop hearts. However, cardiac specification of the mesoderm still occurs in these embryos, likely as the result of overlapping functions of other Bmp family members with Bmp2.

Bmp signaling specifies the cardiogenic lineage, but its effect on the mesoderm is limited to the lateral mesoderm. Why? The reason is that Bmp antagonists and inhibitors are released from midline tissues. The notochord synthesizes and releases chordin and noggin, two proteins that sequester Bmps and prevent binding to their receptors (Fig. 12-2C). If chordin activity is inhibited in cranial paraxial mesoderm, the medial mesoderm has the capacity to form cardiac cells. In addition, the developing neural plate ectoderm releases Wnt1 and Wnt3a, which also antagonize Bmp signaling. If Wnt signaling is abrogated in mouse embryos, multiple hearts are generated. Therefore, because of the antagonizing effects of chordin/noggin and Wnt signaling on Bmp signaling, the influence of Bmp on mesoderm is limited to lateral regions.

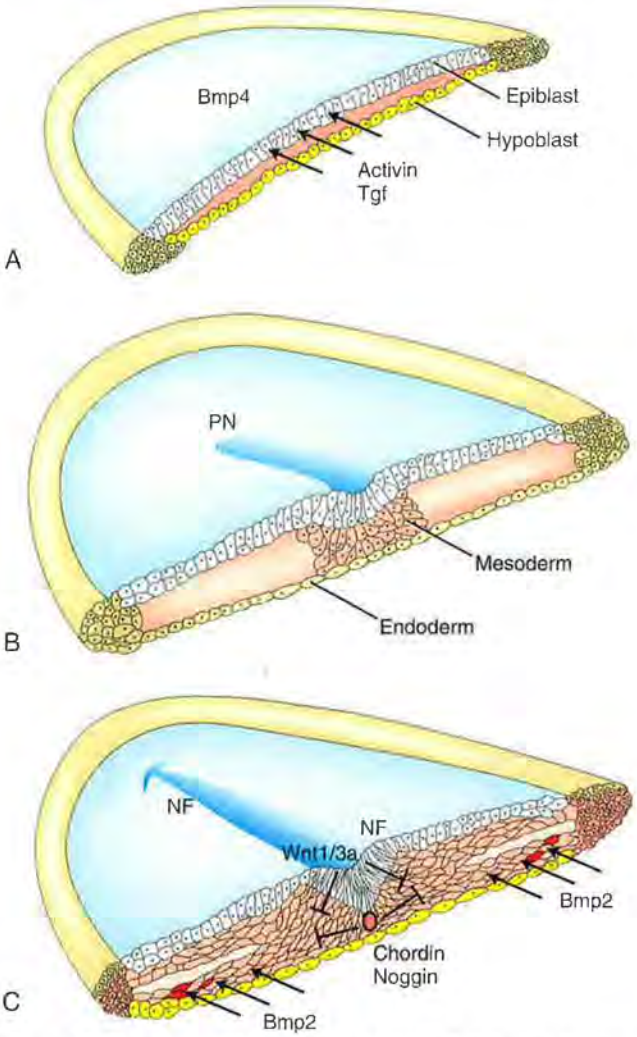


Figure 12-2. Induction of the first heart field. *A, B,* Before and during gastrulation, Tgfβ and activin released by the hypoblast induce cardiogenic potential in a subset of epiblast cells and newly forming mesodermal cells. *C,* Bmps, released from the newly formed endoderm, signal the formation of a cardiogenic lineage from the mesoderm (red cells), but their influence is limited to the lateral mesoderm because of the release of chordin and noggin from the notochord and Wnt1/3a from the forming neuroectoderm. NF, Neural fold; PN, primitive node.

But why is the cardiogenic region limited to the cranial portion of the lateral mesoderm? We know that the caudal lateral plate mesoderm is capable of responding to cardiac specification signals: if it is grafted into the cranial region, it transforms into cardiogenic cells. As covered above, Wnt1/Wnt3a and chordin/noggin inhibit the effects of Bmps on mesoderm. However, other Wnts (e.g., Wnt8) expressed in the cranial and caudal mesoderm also inhibit Bmp effects on the mesoderm. Knowing that Bmp signaling is required for cardiac mesoderm formation, how can Bmp still exert its influence on the cranial lateral mesoderm in the presence of these Wnts but not on the caudal lateral plate? The answer is that other molecules secreted by the cranial *endoderm* antagonize the negative effects of Wnts on Bmp-driven heart formation. These include *secreted* frizzled-like proteins (sFrps) that sequester Wnts and Dickkopfs that bind to and inhibit the Wnt co-receptors of the Lrp (low-density lipoprotein receptor–related protein) class (Fig. 12-3). Hence, in the absence of Wnt signaling, the effect of Bmp is to promote the cardiac lineage in the cranial portion of the lateral mesoderm, whereas in the presence of Wnt signaling, Bmp initiates a blood vessel–forming capacity in the caudal portion of the lateral plate mesoderm. However, recent studies suggest that canonical Wnt signaling has biphasic effects on cardiogenesis depending on the time of action, promoting cardiac specification during gastrulation but later obstructing it. Non-canonical Wnt signaling (Wnt5a and Wnt11) also promotes cardiogenesis.

Several cardiac transcription factors are activated within the first heart field. The earliest transcription factors with limited expression within the cardiac lineage include Nkx2.5, Tbx5, and members of the Gata family. Nkx2.5 is expressed in cardiac progenitor cells soon after the onset of gastrulation under the influence of endodermally derived Bmp. Downstream targets of Nkx2.5 include several other cardiac genes, such as Mef2c, ventricular myosin, and Hand1. A human ortholog of NKX2.5 has been mapped to chromosome 5q35.2, and mutations in this gene are associated with human congenital heart disease, including atrial septal defects, ventricular septal defects, and defects in the conduction system. Nkx2.5 knockout mice die in utero but still form a heart, albeit one without left ventricular markers, with incorrect looping, and with a deranged cranial-caudal identity. So Nkx2.5 expression is not solely responsible for dictating the cardiac cell lineage. Mice null for Gata4 have fewer cardiomyocytes. Mice lacking Gata5 are normal but exhibit elevated Gata4 levels, suggesting a compensatory effect for the loss of Gata5. Gata5 null mice also lacking one of the Gata4 genes exhibit profound cardiac defects, whereas mice with normal Gata5 genes lacking one of the Gata4 genes are normal. This suggests that Gata4 and Gata5 act cooperatively in directing early cardiac lineage. Nkx2.5 and Gatas may mutually reinforce cardiac expression of each other's cardiac expression, as each contains promoter regions for the other.

In summary, the program of early cardiac specification is quite flexible, but it requires the presence of particular morphogens providing a permissive environment for lineage specification. Moreover, no single transcription factor or signaling molecule has been identified that is solely responsible for encoding myocardial specification and differentiation. Rather, it seems that a combination of factors working together is needed to stably specify the cardiac cell lineage.

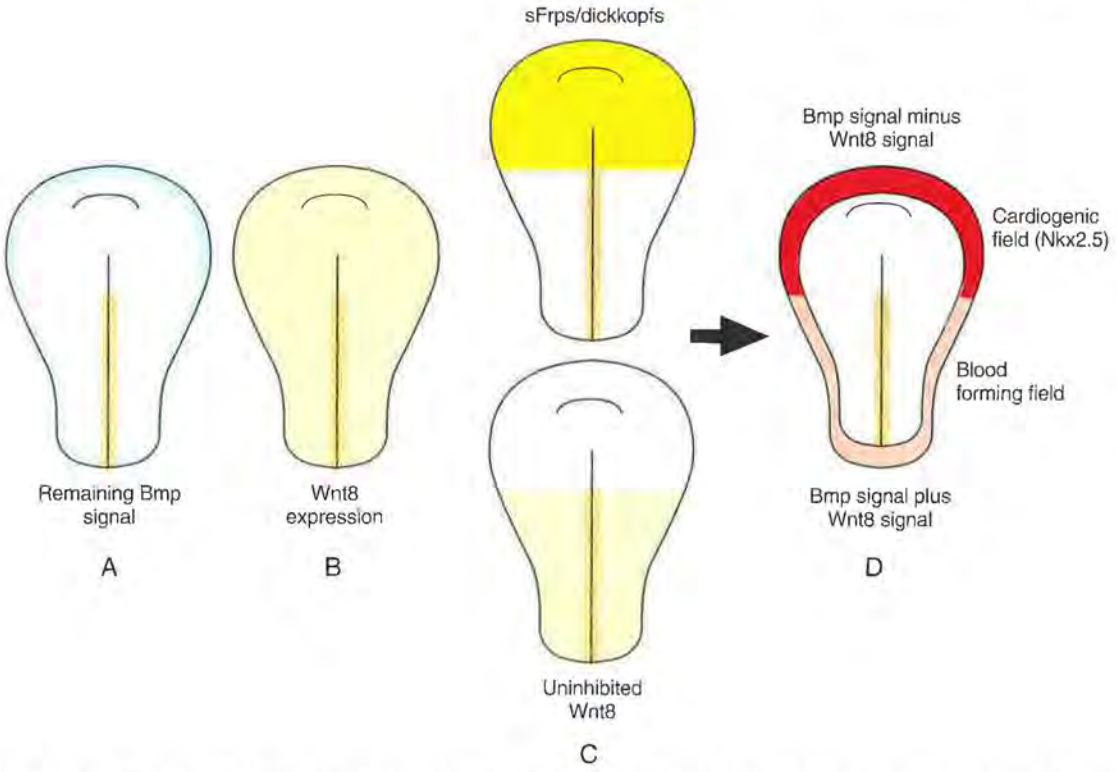


Figure 12-3. Regional specification of cardiogenic mesoderm. *A*, Pattern of Bmp signaling on the mesoderm remaining after accounting for chordin/noggin and Wnt1/3a inhibition. *B*, Pattern of Wnt8 expression in the mesoderm. *C*, Spatial distribution of secreted frizzled-related proteins (sFrps) and dickkopf expression (both Wnt antagonists) in the underlying endoderm, and remaining pattern of uninhibited Wnt8 activity in the mesoderm. *D*, Pattern of expression of the cardiogenic marker Nkx2.5 as a result of Bmp signaling in the absence of Wnt inhibition. In the presence of Bmp and Wnt8 signaling, blood-forming fields are primed.

FORMATION OF PRIMARY HEART TUBE

Animation 12-1: Formation of Primitive Heart Tube. Animations are available online at StudentConsult.

With formation of the intraembryonic coelom, the lateral plate mesoderm is subdivided into somatic and splanchnic layers; the first heart field forms within the splanchnic mesodermal subdivision. During the process of body folding (covered in Chapter 4), the cranialmost portion of the first heart field is pulled ventrally and caudally to lie ventral to the newly forming foregut endoderm (Fig. 12-4). As the lateral body folds move medially, they bring the right and left sides of the first heart field together, and the two limbs of the first heart field fuse at the midline, caudal to the head fold and ventral to the foregut (Fig. 12-5*A-D*). This fusion occurs at the site of the anterior intestinal portal and progresses in a cranial-to-caudal direction as the foregut tube lengthens. As the two limbs of the first heart field fuse, a recognizable pair of vascular elements called the **endocardial tubes** develops within each limb of the first heart field (Fig. 12-5*B, C*). These vessels form within the first heart field from a seemingly distinct progenitor population from other endothelial subtypes through mechanisms that still are not well understood. The cells of the endocardial tubes coalesce into a single tube as the limbs of the first heart field join to make the primary heart tube (Fig. 12-5*C, D*). If fusion of the first heart field limbs fails, two tube-like structures form rather than a single primary heart tube,

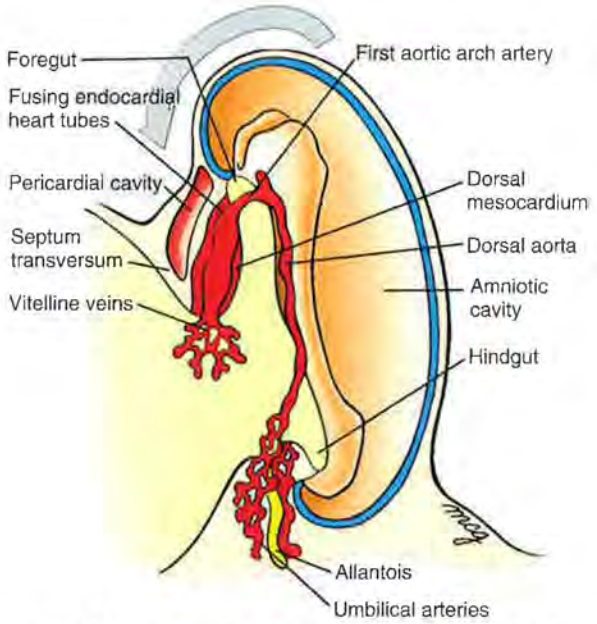
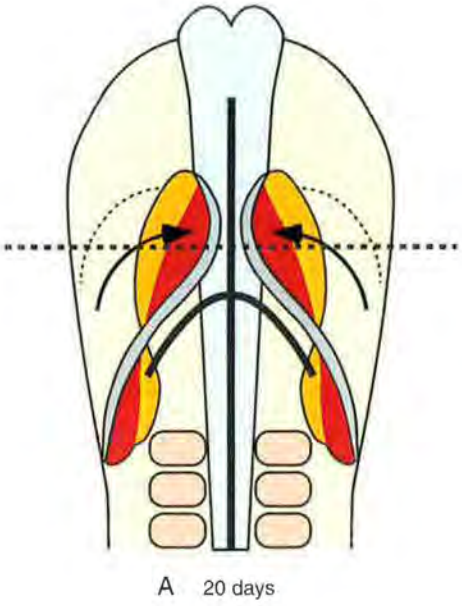
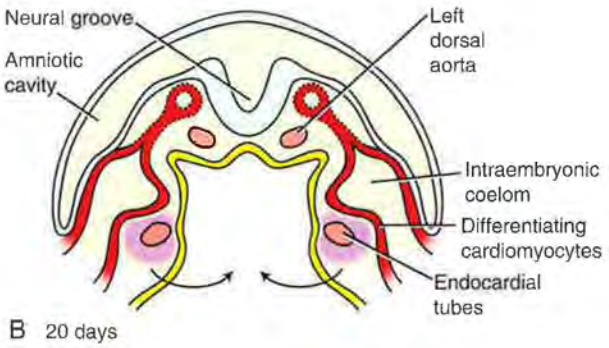
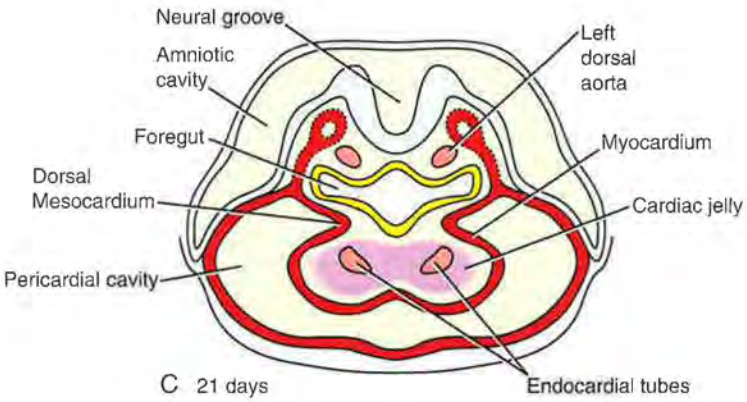


Figure 12-4. Formation of the first aortic arch artery and dorsal aorta during the third week. The paired dorsal aortae develop in the dorsal mesoderm on either side of the notochord and connect to the fusing endocardial heart tubes while body folding ensues. As flexion and growth of the head fold (large curved arrow) carry the forming primary heart tube into the cervical and then into the thoracic region, the cranial ends of the dorsal aortae are pulled ventrally until they form a dorsoventral loop—the first aortic arch artery. A series of four more aortic arch arteries will develop during the fourth and fifth weeks.







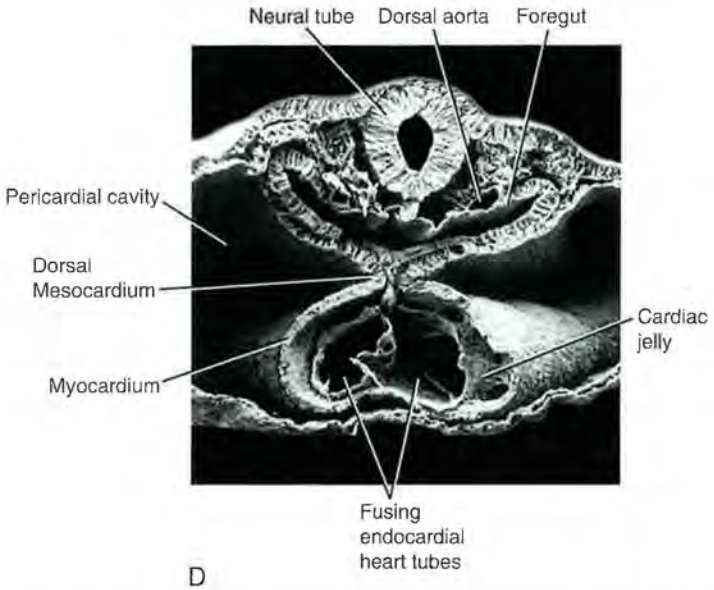


Figure 12-5. Formation of the primary heart tube. During the process of body folding in the third week, the cranialmost portion of the first heart field is pulled ventrally and caudally to lie beneath the newly forming foregut. *A,* Ventral view; dashed horizontal line indicates the level of sections illustrated in *B* and *C;* curved solid line indicates the anterior (cranial) intestinal portal of the developing foregut; vertical solid line indicates the notochord; red, first heart field; orange, second heart field. As the lateral body folds (arrows) fuse in the midline in a cranial-to-caudal progression, they also bring the right and left sides of the first heart field together (red). *B, C,* Drawings of cross sections at the level indicated by the dashed line in *A,* with *C* at a later stage than *B. D,* Scanning electron micrograph of a cross section. As the two limbs of the first heart field fuse, a recognizable pair of vascular elements called the endocardial tubes develops within each limb of the first heart field. These endocardial tubes then fuse to form the primary heart tube.

Case 2:12-md-02342-CMR Document 1561-22 Filed 03/01/16 Page 11 of 23

Figure 12-6. Scanning electron micrographs of developing mouse embryos. *A-C*, Head folding progressively translocates the developing endocardial tubes from a region initially just cranial to the neural plate to the thoracic region (arrow in *A*, cardiogenic region).

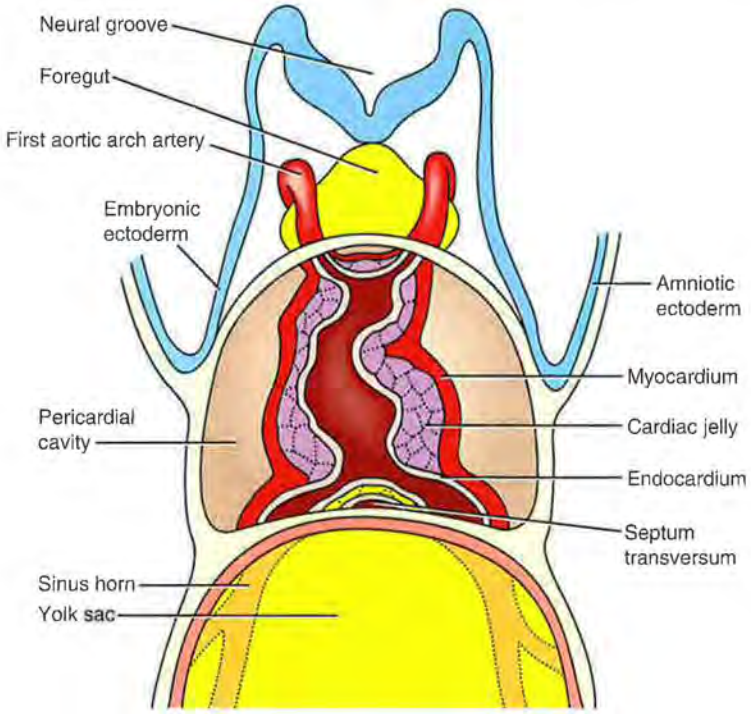


Figure 12-7. Composition of the primary heart tube walls. By twenty-two days, the endocardium of the primary heart tube is invested by an acellular layer of cardiac jelly and a layer of myocardial cells. The myocardium is derived from a mass of splanchnic mesoderm that encloses the endocardial heart tube. The myocardium then secretes the extracellular cardiac jelly between itself and the endocardium.

leading to **cardia bifida** (however, both tubes persist, contract, and continue to undergo cardiogenesis, including looping; looping is covered below). The primary heart tube harbors progenitors for the atria and left ventricle, as well as endocardium. As the fusion process continues, cell proliferation in the first heart field continues to add the more caudal segments of the heart, including the atrioventricular canal, the primitive atria, and a portion of the sinus venosus (covered later in the chapter). Late in the third week, cranial body folding brings the developing heart tube into the thoracic region (Figs. 12-4, 12-6; also covered in Chapters 4 and 11).

By the twenty-first to twenty-second day, the primitive endocardial tube is surrounded by a mass of splanchnic mesoderm containing myocardial progenitors that aggregate around fused endocardial tubes to form the **myocardium**. A thick layer of acellular extracellular matrix, the **cardiac jelly**, is deposited mainly by the developing myocardium, separating it from the endocardial tube (Fig. 12-7). The **epicardium** (visceral lining of the pericardial cavity covering the heart) is formed later by a population of mesodermal cells that are independently derived from splanchnic mesoderm that migrates onto the outer surface of the myocardium (covered later in the chapter).

A series of constrictions and expansions develop in the primary heart tube (Fig. 12-8). Over the next five weeks, as the tubular heart lengthens, these expansions contribute to the various heart chambers. Starting at the caudal (inflow) end, the **sinus venosus** consists of the partially confluent left and right **sinus horns**, into which the common cardinal veins (covered later in the chapter)

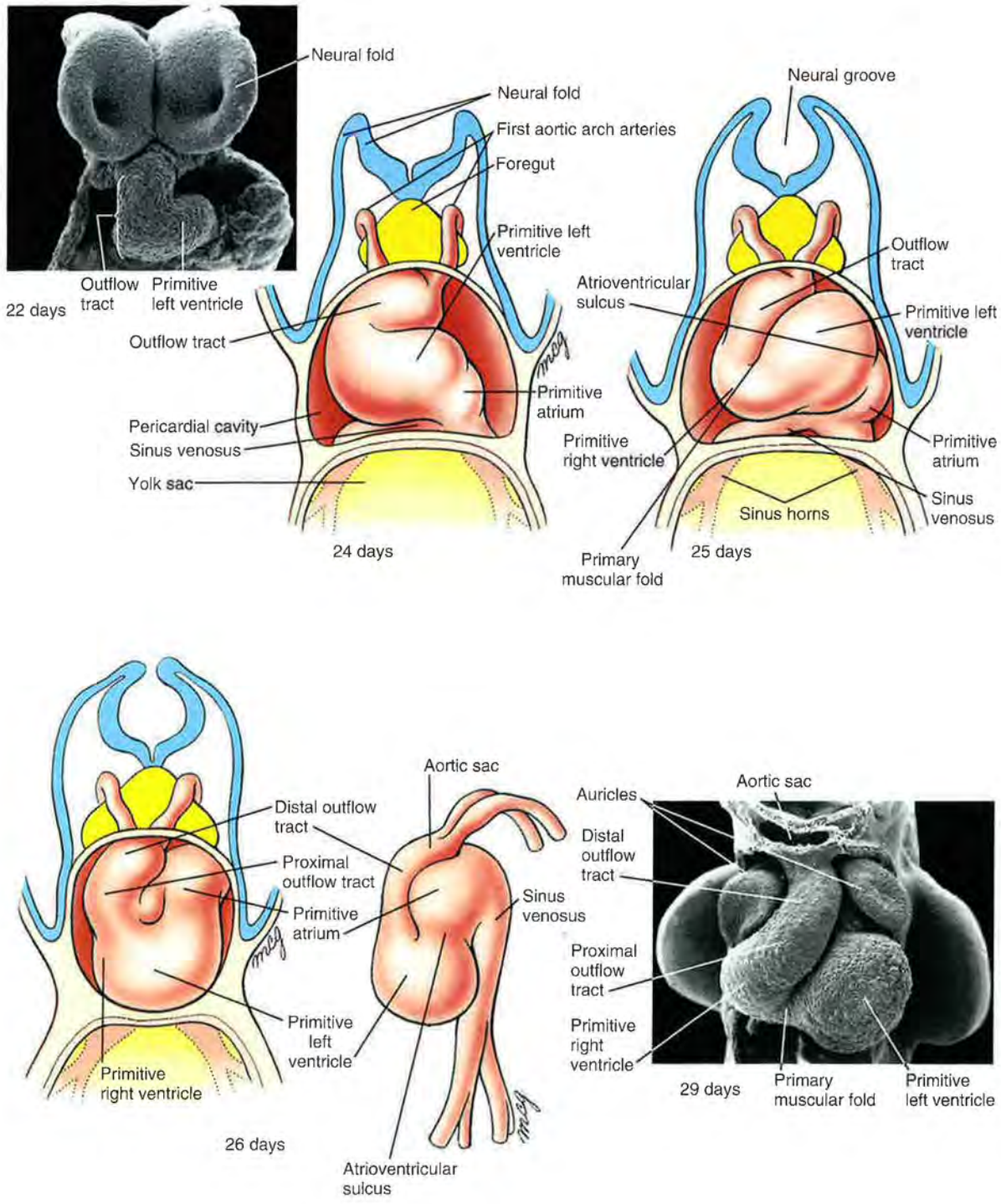


Figure 12-8. Regionalization of the heart tube during its lengthening. As the heart tube lengthens and adds to the outflow segment, looping of the heart tube repositions the outflow tract ventrally and to the right, shifts the primitive left ventricle to the left, and shifts the primitive atrium dorsally and cranially. Addition of myocardium at the arterial end forms the right ventricle and the future proximal and distal segments of the outflow tract. The primitive left ventricle will form the definitive left ventricle, and the primitive atrium will give rise to a portion of the atrial wall and auricles of the heart. During this process, deepening external folds and grooves increasingly distinguish each segment of the heart tube.

drain. Cranial to the sinus venosus, the next chamber is the **primitive** (or **common**) **atrium**, which, as a result of the subsequent formation of the atrial septal complex, eventually becomes divided into the right and left atria. Connected in series with the atrium are the **atrioventricular canal**, the **primitive left ventricle**, and the developing **primitive right ventricle** and **outflow tract**. The primitive left ventricle is separated from the primitive right ventricle by a **primary muscular fold** (formerly referred to as the **bulboventricular fold**), the latter contributing to the **muscular ventricular septum**. Whereas the atria, atrioventricular canal, and left ventricle are largely derived from the first heart field, the right ventricle and outflow tract are not. Rather, they are derived from an additional source of cardiac precursor cells, referred to as the **second heart field**. The outflow tract forms the outflow region for both the left and right ventricles. The outflow tract can be subdivided into a **proximal outflow tract** (conus arteriosus), which eventually becomes incorporated into the left and right ventricles, and the **distal outflow tract** (truncus arteriosus), which eventually splits to form the ascending aorta and pulmonary trunk. The distal outflow tract is connected at its cranial end to a dilated expansion called the **aortic sac**. The aortic sac is continuous with the first aortic arch artery and, eventually, is continuous with the other four aortic arch arteries as they develop. The aortic arch arteries form major arteries transporting blood to the head and trunk (covered in Chapter 13).

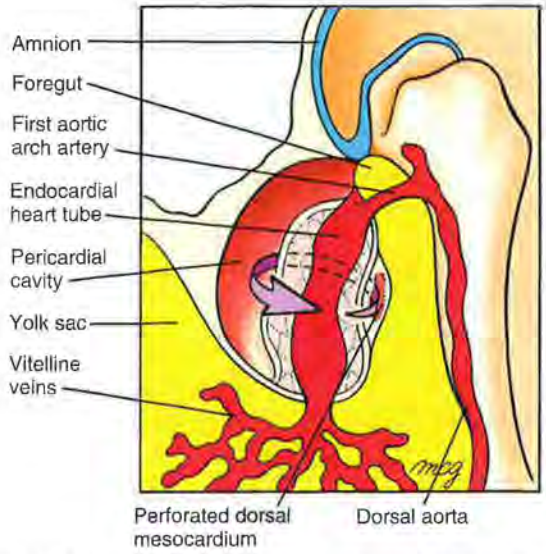


Figure 12-9. Formation of the transverse pericardial sinus of the definitive pericardial cavity by rupture of the dorsal mesocardium early in the fourth week. Arrow passes through the transverse pericardial sinus.

The primary heart tube is initially suspended in the developing pericardial cavity by a **dorsal mesocardium (dorsal mesentery of the heart)** formed by splanchnic mesoderm located beneath the foregut. Subsequently, this dorsal mesocardium ruptures over almost the entire length of the heart tube, with the exception of the caudalmost aspect, where a small but very important component of the dorsal mesocardium persists. As a result, the heart is left suspended in the pericardial cavity by its developing arterial and venous poles, with the region of the ruptured dorsal mesocardium becoming the **transverse pericardial sinus** within the pericardial sac of the definitive heart (Fig. 12-9). Ligatures sometimes are passed through this space and around the vessels at either pole to control blood flow in children or adults undergoing surgery.

As noted earlier, not all of the cardiac cells found in the mature heart are derived from the first heart field. Rather, additional sources of cardiogenic precursors are recruited from the mesoderm immediately adjacent and medial to the initial cardiac crescent (Fig. 12-10). While the developing primary heart tube continues to expand, there is continued recruitment of cardiac progenitor cells from outside the original first heart field at both the arterial (cranial) and venous (caudal) poles. The source of

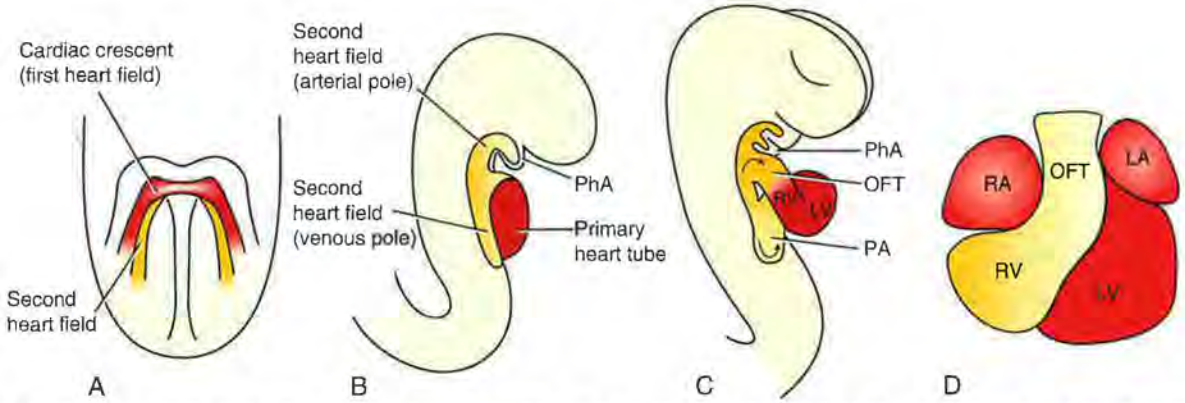


Figure 12-10. The second source of cardiogenic progenitors for the heart, the second heart field (shown in orange in *A-D*). *A*, Location of the second heart field relative to the first heart field before body folding. The second heart field is located within the splanchnic mesoderm just medial and slightly caudal to the first heart field (first heart field shown in red). *B*, After formation of the primary heart tube, the second heart field becomes located dorsal to the dorsal mesocardium and runs along the craniocaudal axis. *C*, With rupture of the dorsal mesocardium, the second heart field is divided into a caudal segment, responsible for adding to the venous pole of the heart, and a cranial segment, responsible for lengthening the heart tube at the arterial pole. *D*, Ventral view of the looped heart shows the contributions of the first and second heart fields (contributions of the second heart field to the atria are not visible in this view). LA, Left atrium; LV, left ventricle; OFT, outflow tract; PA, primitive atrium; PhA, pharyngeal arch; RA, right atrium; RV, right ventricle.

these cells is referred to as the **second heart field**. The primitive heart tube lengthens at both ends, particularly the outflow (arterial) end, through the addition of cardiac progenitors from the second heart field mesoderm. Lineage-tracing studies suggest that in mammals the proximal and distal outflow tract, the right ventricle, and a portion of the venous pole and atria are derived from the second heart field mesoderm (Fig. 12-10*D*).

In the Research Lab

ROLE OF SECOND HEART FIELD IN FORMATION OF OUTFLOW SEGMENT OF HEART

Animation 12-2: Contributions of First and Second Heart Fields.
Animations are available online at StudentConsult.

Cells of the first and second heart fields may arise from a common precursor established before the cardiac crescent stage (likely during early gastrulation). Recent studies suggest that progenitors for the second heart field lie just medial and slightly caudal to the first heart field within the lateral plate mesoderm (see Figs. 12-1, 12-10). Like the first heart field, the second heart field is subjected to the influences of Bmps and Fgfs released by the foregut (pharyngeal) endoderm that activate cardiogenic transcription factors. However, the more medial location of the second heart field at the cardiac crescent stage also positions these cells closer to the negative influence of Wnts and chordin/noggin emanating from the developing notochord and neural plate (see Figs. 12-2, 12-10). Manifestation of the cardiac cell lineage within the second heart field is likely suspended until the primary heart tube is formed and the intervening distance between the second heart field and the midline neural tube/notochord is increased. Therefore, cells of the second heart field may not represent a distinct cardiogenic lineage from the first heart field.

As the two limbs of the cardiac crescent move toward the midline during fusion, the second heart field cells come into contact with the dorsal surface of the primary heart tube (future inner curvature of the heart) and end up at both the cranial and caudal ends of the developing dorsal mesocardium (see Fig. 12-10*B, C*). It is after the second heart field cells come to lie ventral to the foregut that expression of Nkx2.5 and Gata4 increases in the second heart field (Fig. 12-11). Second heart field cells lying just cranial to the arterial outflow of the heart tube and ventral to the developing pharyngeal endoderm assume a *right* ventricular identity, whereas cells more caudal to the arterial outflow contribute to the wall of the proximal and distal outflow tracts. Those at the inflow end of the heart tube contribute myocardial cells to the wall of the atria, atrial septum, and sinus venosus. The bulk of heart tube lengthening comes from proliferation within the second heart field at the arterial pole.

GENE MUTATIONS TARGET FIRST AND SECOND HEART FIELDS

Mutations in particular genes reveal regional sensitivities of the myocardium that reflect the origin of their cardiomyocyte progenitors. For instance, in Tbx5-deficient mice (Tbx5 is a T-box transcription family member that is expressed in the primary heart tube), the atrium is abnormal and the left ventricle is hypoplastic. Yet, the right ventricle and outflow tract seem normal, suggesting that this mutation mainly targets proliferation and development of cells of the first heart field. Isl1 is expressed in the second heart field. Mice null for Isl1 typically develop only two heart chambers: the atria and the left ventricle. The outflow tract is missing, right ventricular markers are not expressed, and the posterior atrial myocardium is hypoplastic. Fgf8 is expressed in the ectoderm and pharyngeal endoderm near the arterial pole of the heart tube. Proper Fgf8 signaling within the second heart field is necessary for continued proliferation of cranial second heart field cells at the arterial pole. Fgf8 hypomorphs (an embryo with a partial loss-of-function mutation; i.e., Fgf8 expression in the hypomorph is knocked down but is not eliminated completely) die as a result of abnormal outflow tract development. Tbx1 (lost in 22q11.2 deletion syndrome), a transcription factor expressed in the second heart field, interacts genetically with Fgf8. Again, loss of Tbx1 expression in the second heart field reduces myocardial cell number in the outflow tract and right ventricle, whereas forced Tbx1 overexpression in the second heart field causes an expansion of the outflow tract. From these studies, it is clear that in addition to a first heart field, a large portion of the definitive heart tube arises from the second heart field. Several other signaling molecules and transcription factors play important roles in mediating continued proliferation or survival of second heart field cells, including Shh, canonical Wnts, Pdgf, retinoic acid and retinoic acid receptors, Mef2c, Msx1, Msx2, Hand2, Tbx18, Shox2, Foxa2, Foxc1, and Foxc2. Once heart tube elongation is completed, studies suggest that cranial second heart field mesodermal cells in the pharyngeal arches may activate a branchiomeric skeletal muscle program (covered in Chapter 17). Lengthening of the heart tube by the second heart field plays an important role in proper cardiac looping and septation of the heart.

CARDIAC LOOPING

Animation 12-3: Looping of Primitive Heart Tube.
Animations are available online at StudentConsult.

On day 23, the primary heart tube begins to elongate and simultaneously bend into a C-shaped structure, with the bend extending toward the right side. Formation of this bend is not simply a matter of forming a kink in the tube, with the right side of the tubular heart becoming the outer curvature and the left side forming the inner curvature. Rather, it seems that the ventral surface of the primary heart tube forms the right outer curvature of the C-shaped heart, because the ventral surface is displaced toward the right by torsional forces working along the craniocaudal axis (Fig. 12-12). With the rupture of the dorsal mesocardium, much of the dorsal side of the straight primary heart tube becomes situated on the inner curvature of the C-shaped heart. As the heart tube continues to elongate at both arterial and venous poles, it takes on an S-shaped configuration. In the process, the primitive right ventricle is displaced caudally, ventrally, and to the right; the developing primitive left ventricle is displaced to the left. The primitive atrium acquires a more dorsal and cranial position (Fig. 12-13; see also Fig. 12-8). By day twenty-eight, the elongation of the heart tube is complete, but there continues to be additional remodeling such that the outflow tract comes to lie between the presumptive future atria, and the atrioventricular canal aligns with both ventricles (see Fig. 12-8). The end result of cardiac looping is to bring the four presumptive chambers of the future heart into their correct spatial relationship to each other. The remainder of heart development consists mostly of remodeling these chambers, developing the appropriate septa and valves between them, and forming the epicardium, coronary vasculature, and cardiac innervation and conducting system.

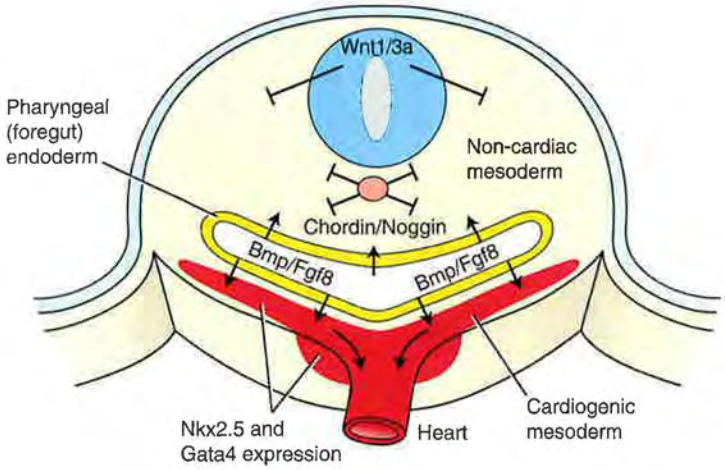


Figure 12-11. Role of growth factors in lengthening of the heart tube by the second heart field. Specification of cardiogenic precursors in the second heart field is similar to that in the first heart field. The cardiogenic promoting effect of Bmp and Fgf8 released by the endoderm on the splanchnic mesoderm is no longer antagonized after formation of the foregut by Wnts and chordin/noggin released by midline tissues. As a result, the cardiogenic mesoderm of the second heart field begins to express cardiac markers (e.g., Nkx2.5 and Gata4), proliferates, and drives the lengthening of the heart tube.

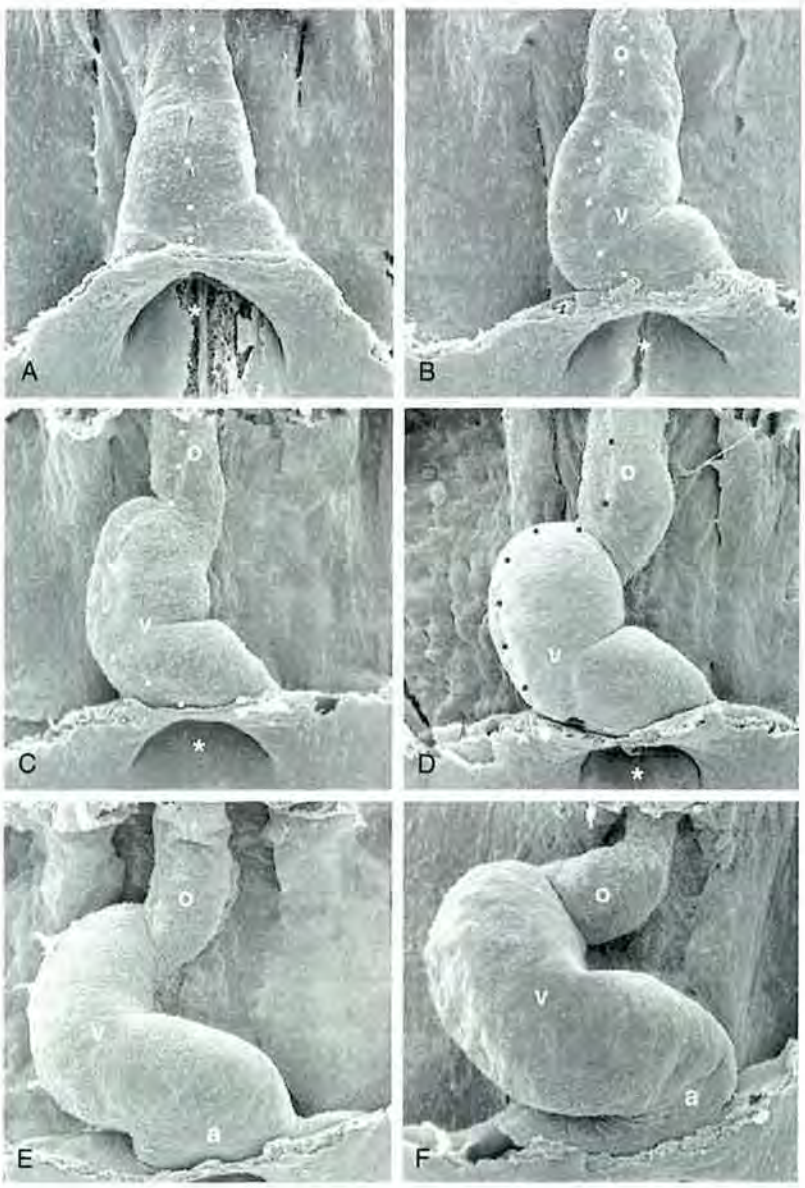


Figure 12-12. Scanning electron micrographs showing looping of the chick heart tube in ventral views (endoderm has been removed). *A, B,* Shows the primary heart tube just before overt looping. The ventral midline of the primary heart tube is marked by the dotted line. *C-F,* Cardiac looping is driven in part by cardiac lengthening from the second heart field. Note that looping to the right is accompanied by twisting, such that the original ventral surface of the primary heart tube becomes the outer curvature of the looped heart. These forces help drive the formation primary muscular fold. Several of the cardiac regions are easily identifiable during this process, including atrium (a), outflow tract (o), and primitive left ventricle (v). The asterisks demarcate the anterior intestinal portal.

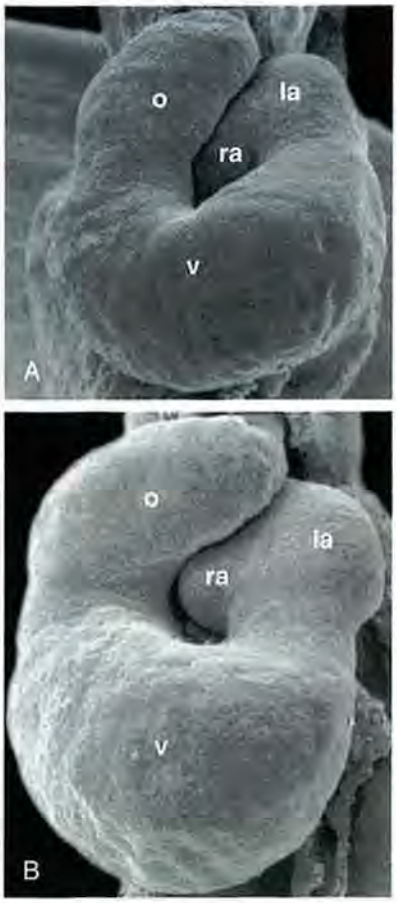


Figure 12-13. Scanning electron micrographs showing late views of looping of the chick heart tube. *A, B,* Position and morphology of the heart regions at progressively later end stages of cardiac looping. Ventral view of the outflow tract (o), non-septated ventricle (v), and non-septated right atrium (ra) and left atrium (la) showing their relative anatomical positions near the end of cardiac looping. Note that both atrial and venous poles are now adjacent to each other and that the outflow tract is moving leftward and ventral to the atria.

In the Clinic

Sidedness in Heart Looping

As covered in Chapter 3, abnormal left-right axis determination can lead to development of **heterotaxy** (with an estimated incidence of 3 out of 20,000 live births). This term is sometimes used to describe any defect ascribed to abnormal left-right axis formation, be it a reversal of some organs **(partial situs ambiguus)** or a reversal of all viscera **(situs inversus totalis)**. With regard to the heart, this may include abnormal looping, resulting in **ventricular inversion** (Fig. 12-14). Proper looping toward the right is a prerequisite for proper cardiac septation, as it is required to bring the primitive left ventricle toward the left, the primitive right ventricle toward the right, and the outflow tract region to the middle. Because individuals with situs inversus totalis exhibit a reversal in handedness of all organs, they exhibit few problems. In contrast, **visceroatrial heterotaxy syndrome** in humans (where the abdominal viscera and the atrial pole are oriented on opposing sides) is associated with structural defects, including a common atrium, malalignment of atrioventricular canal and outflow tract, and abnormal venous and arterial vascular connections.

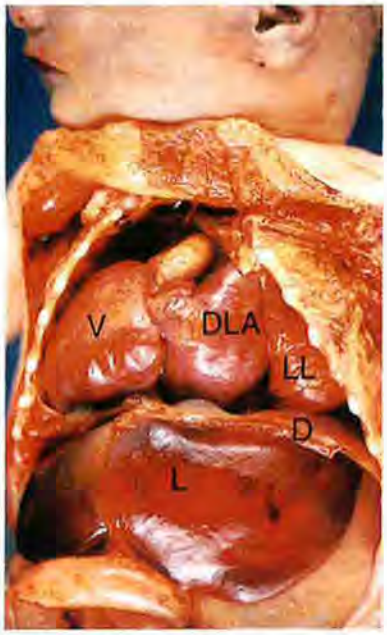


Figure 12-14. Infant with ventricular inversion, a condition in which the looping of the heart tube is reversed from its normal sinistral pattern, producing a heart that has its apex inferior and to the right (rather than left) side. D, Diaphragm; DLA, dilated left atrium; L, liver; LL, left lung; V, ventricle.

Besides inverted situs, indeterminate left-right axis formation can lead to bilateral left-sidedness or right-sidedness, so-called isomerism. For example, in the condition called right atrial isomerism, both atria have right atrial morphology. Similarly, in left pulmonary isomerism, both lungs have the lobar and hilar anatomy of the left lung.

In the Research Lab

Mechanisms Driving Cardiac Bending and Looping

Cardiac looping involves two major processes: establishing the directionality of looping, and performing the biomechanical steps that drive the looping itself. Directionality of looping reflects the left-right asymmetry established early during gastrulation (covered in Chapter 3), which is superimposed on the morphogenetic mechanisms of cardiac looping. In fact, the initial bending of the heart tube into the C shape is the first morphological evidence of embryonic left-right asymmetry.

The precise mechanisms driving the initial bending and the heart tube's continued looping into an S-shaped tube are still unclear, even though considerable effort has gone into identifying the forces responsible for the process. At one time, it was suggested that these processes occur simply because the heart tube, being anchored on both ends, outgrows the length of the primitive pericardial cavity and is forced to bend and loop. However, hearts excised from experimental animals and grown in culture demonstrate an intrinsic ability to bend, likely due to active changes in cell shape caused by forces of actin polymerization. Still, excised hearts do not exhibit rightward torsion, suggesting that forces external to the primary heart tube drive the latter. Experimental manipulations in chick embryos suggest that asymmetrical growth within

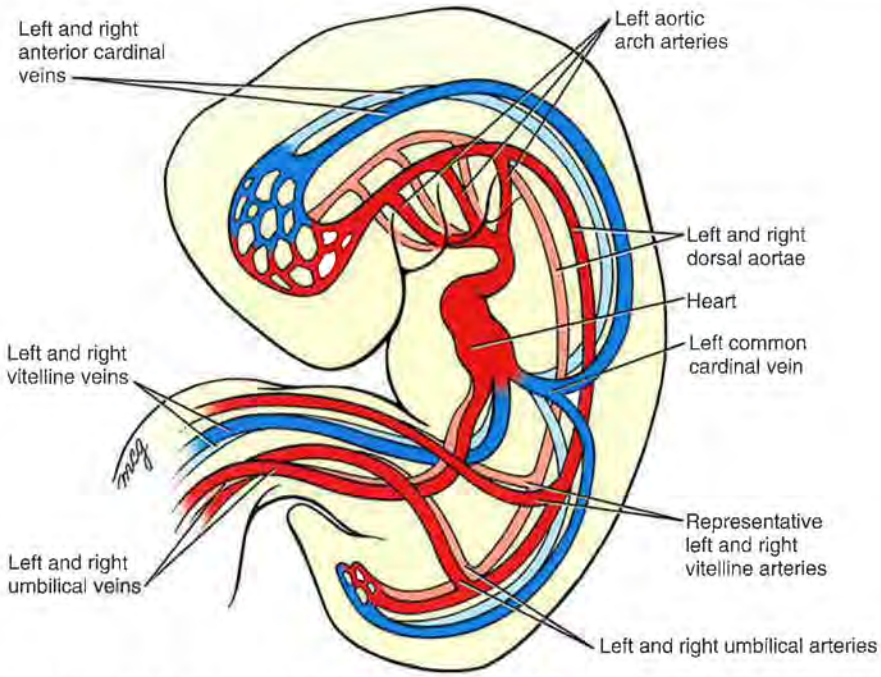


Figure 12-15. Schematic depiction of the embryonic vascular system in the middle of the fourth week. The heart has begun to beat and to circulate blood. The outflow tract is now connected to three pairs of aortic arch arteries and the paired dorsal aortae that circulate blood to the head and trunk. Three pairs of veins—umbilical, vitelline, and cardinal—deliver blood to the inflow end of the heart.

the primitive atria and the attached ventral splanchnopleure provide the torsional forces driving displacement of the heart to the right, while the dorsal mesocardium constrains this motion on the dorsal side, thereby resulting in the C-shaped bend. Other models suggest that remnants of the dorsal mesocardium shorten and force the heart tube to bend. However, the primary heart tube exhibits signs of bending before rupture of the dorsal mesocardium. Alternatively, before rupture, the dorsal mesocardium may exert tension on the future inner curvature, providing the biomechanical driving force necessary for bending. Finally, asymmetrical cell proliferation and growth within the cranial second heart field may also generate torsional forces necessary for generating the C-shaped bend.

FORMATION OF PRIMITIVE BLOOD VESSELS ASSOCIATED WITH THE ENDOCARDIAL TUBE

Many of the major vessels of the embryo, including the paired dorsal aortae, develop at the same time as the endocardial tube. The inflow and outflow vessels of the future heart make connections with the endocardium of the primary heart tube even before this tube is translocated into the thorax. The paired **dorsal aortae**, which form the primary outflow vessels of the heart, develop in the dorsal mesenchyme of the embryonic disc on either side of the notochord. As the flexion and growth of the head fold carry the heart tube into the cervical and then thoracic region, the cranial ends of the dorsal aortae are pulled ventrally until they form a dorsoventral loop—the **first pair of aortic arch arteries**. (Fig. 12-15; see also Figs. 12-4, 12-7, 12-8, 12-9). A series of four more aortic arch arteries develop during the fourth and fifth weeks in connection with the mesenchymal pharyngeal arches (covered in Chapters 13 and 17). In addition, the craniocaudal flexure facilitates cardiac looping by bringing the venous (sinus venosus) and arterial (distal outflow tract and aortic sac) poles closer to one another in a process called **convergence**.

Six vessels, three on each side (Fig. 12-15), initially provide the inflow to the heart. Venous blood from the body of the embryo enters the heart through a pair of short trunks, the **common cardinal veins**, which are formed by the confluence of the paired **posterior cardinal veins** draining the trunk and the paired **anterior cardinal veins** draining the head region (see Fig. 12-15). A pair of **vitelline veins** drains the yolk sac, and a pair of **umbilical veins** delivers oxygenated blood to the heart from the placenta. The embryonic venous system is discussed in Chapter 13.

In the Research Lab

SUBREGIONS OF HEART ARE SPECIFIED EARLY IN DEVELOPMENT

The chambers of the heart are developmentally, electrophysiologically, and pharmacologically distinct. How does this regionalization develop within a single heart tube? Fate mapping studies show that cardiac progenitor cells within the epiblast are topologically organized such that the cardiac inflow progenitors are located more lateral, and the outflow progenitors

Case 2:12-md-02342-CMR Document 1561-22 Filed 03/01/16 Page 18 of 23

more medial. Subsequently, during the process of gastrulation, this orientation is converted to a craniocaudal (arterial/venous) topography by the time of the cardiac crescent stage. Cells within the first heart field are still plastic with regard to chamber specification: if caudal cardiac progenitor tissue is substituted for cranial cardiogenic tissue, proper hearts are generated. However, soon afterward, commitment to particular chambers is evident by the expression of chamber-specific regulators.

Regionalization of the heart is likely an outcome of having at least two separate heart areas within the first heart field. In mice, clonal analysis suggests that the atrial region becomes clonally distinct (i.e., clones of progenitor cells become restricted to a single compartment) before the rest of the heart. Tbx5 has been linked to atrial lineage determination. Initially expressed in the entire first heart field, Tbx5 expression becomes limited to the sinus venosus and atria, with some expression in the left ventricle (i.e., first heart field derivatives; Fig. 12-16). Tbx5 knockout mice exhibit severe hypoplasia of these chambers, whereas forced expression of Tbx5 throughout the heart leads to loss of ventricular-specific gene expression, essentially "atrializing" the heart. Mutations in human TBX5 have been identified in families with **Holt-Oram syndrome**, which includes heart chamber malformations, atrial septal defects, and cardiac conduction system anomalies. Irx4, an Iroquois homeoprotein, is expressed only in the cranial portion of the first heart field (Fig. 12-16); later, it is restricted to ventricular cells, where it stimulates the expression of ventricular myosin heavy chain-1 (Mhc1v) and suppresses atrial myosin heavy chain-1 (Mhc1a). Irx4 is thought to maintain the cranial-caudal phenotype of the heart by suppressing atrial commitment, because loss of Irx4 expression in mice leads to ectopic expression of atrial markers in the ventricles. Once the initial heart tube begins to lengthen and cardiac bending and looping begin, major changes occur in the expression of several chamber/region-restricted transcription factors, with the expression of a number of genes becoming increasingly restricted to atrial, atrioventricular, ventricular, and outflow tract regions. For example, Tbx20 encodes a transcription factor with heart chamber–promoting characteristics. Tbx20 negatively regulates Tbx2, a transcription factor normally expressed in non-chamber myocardium, such as the wall of the atrioventricular canal and outflow tract, by sequestering receptor-mediated Smad signaling. Hence, Tbx20 and Tbx2 work in concert to delineate chamber from non-chamber myocardium along the cardiac tube.

Expression of several of the chamber-specific properties depends on many of the same cranial/caudal-patterning influences driving regionalization of the neural ectoderm and paraxial mesoderm. Application of excess retinoic acid during early chick embryo cardiogenesis causes "atrialization" or "caudalization" of the primitive heart tube, as indicated by ubiquitous expression of Tbx5 throughout the heart tube, whereas treatment with retinoic acid antagonists leads to "ventricularization." Atrial gene expression in mice is similarly expanded with retinoic acid treatments in utero. A potential mechanism for localized retinoid signaling in embryos is the restricted expression of retinaldehyde dehydrogenase-2 (Raldh-2), a limiting enzyme in retinoic acid biosynthesis. Restricted expression of Raldh-2 to the caudal border of the cardiogenic field is correlated with the caudal limit of atrial gene expression in both chick and mouse embryos. Mouse embryos deficient in Raldh-2 have reduced Tbx5 expression in the caudal heart field, lack atria and limbs, and die in utero. Recent studies support the hypothesis that retinoic acid plays an essential role in establishing the caudal boundary of the cardiogenic field.

COORDINATED REMODELING OF HEART TUBE AND PRIMITIVE VASCULATURE PRODUCES SYSTEMIC AND PULMONARY CIRCULATIONS

At day twenty-two, the primitive circulatory system is bilaterally symmetrical: right and left cardinal veins (common, anterior, and posterior) drain the two sides of the body, and blood from the heart is pumped into right and left aortic arches and dorsal aortae. The

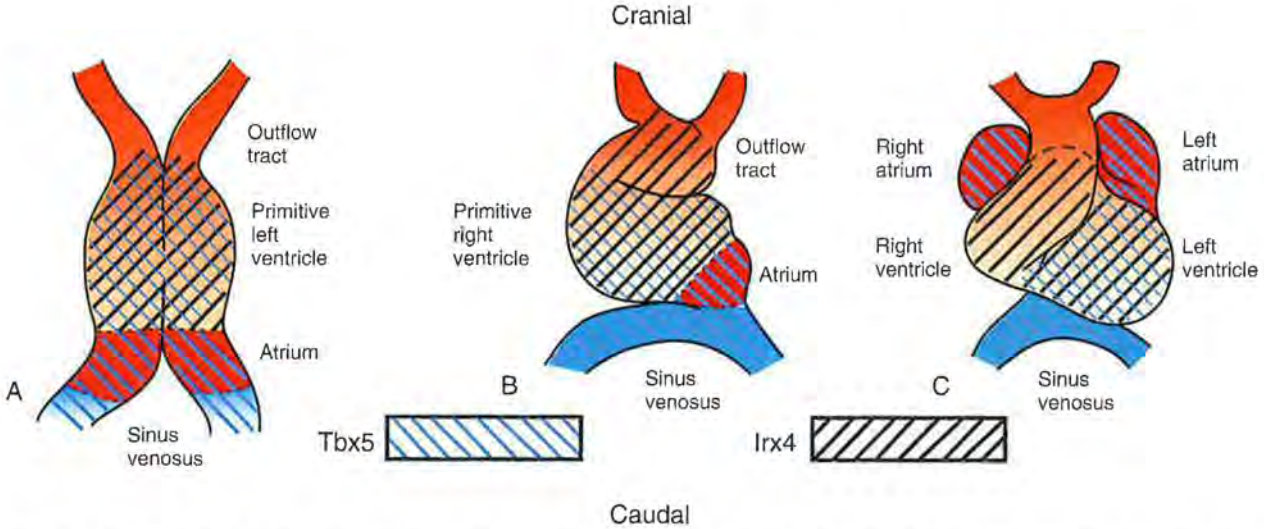


Figure 12-16. Schematic depiction of expression patterns of the transcription factors Tbx5 and Irx4 during early specification of the cardiac chambers. *A-C,* Ventral views at three stages of heart development. Tbx5, linked to the atrial phenotype, becomes increasingly restricted to the atria and the sinus venosus, whereas Irx4, a transcription factor that drives Mhc1v expression and suppresses Mhc1a expression, becomes increasingly restricted to ventricular cells. The thickness of the lines indicates the levels of gene expression, with thicker lines representing higher levels of expression than thinner lines.

paired dorsal aortae fuse at axial levels T4 to L4 during the fourth week to form a single midline dorsal aorta. The venous system undergoes a complicated remodeling (detailed in Chapter 13), with the result that all systemic venous blood drains into the right atrium through the newly formed superior and inferior caval veins.

The heart starts to beat on day twenty-one, and by day twenty-four to twenty-five, blood begins to circulate throughout the embryo. Venous return initially enters the right and left sinus horns via the common cardinal, umbilical, and vitelline veins (Fig. 12-17). Within the next few weeks, the venous system is remodeled so that all systemic venous blood enters the right sinus horn via the **superior** and **inferior caval veins** (Fig. 12-17). As venous inflow shifts to the right, the left sinus horn ceases to grow and is transformed into a small venous sac on the posterior wall of the heart (see Fig. 12-17). This structure gives rise to the **coronary sinus** and the small **oblique vein of the left atrium**. The coronary sinus will receive most of the blood draining from the coronary circulation of the heart.

As the right sinus horn and the caval veins enlarge to keep pace with the rapid growth of the rest of the heart, the right side of the sinus venosus is gradually incorporated into the right caudal/dorsal wall of the developing atrium, displacing the original right half of the primitive atrial wall farther to the right (Figs. 12-17, 12-18). The differential growth of the right sinus venosus also repositions the vestigial left sinus horn (the future coronary sinus) to the right. The portion of the atrium consisting of the incorporated sinus venosus is now called the **sinus venarum**. The original right side of the primitive atrium can be distinguished in the adult heart by the pectinate (comb-like) trabeculation of its wall, which contrasts with the smooth wall of the sinus venarum.

Through a process of **intussusception** (folding in of an outer layer) of the right sinus venosus, the openings, or **ostia**, of the superior and inferior caval veins and future coronary sinus are incorporated into the dorsal wall of the definitive right atrium, where they form the **orifices of the superior and inferior caval veins** and the **orifice of the coronary sinus** (Fig. 12-18*B*). As this occurs, a pair of tissue flaps, the **left** and **right venous valves**, develops on either side of the three ostia (see Fig. 12-18*B*). Cranial to the sinuatrial orifices, the left and right valves join to form a transient septum called the **septum spurium**, which, along with the left venous valve, becomes part of the septum secundum, one of the septa contributing to the separation of the definitive right and left atria (covered later in the chapter). The right venous valve persists and contributes to the formation of the **terminal crest (crista terminalis)**, the **valve of the inferior caval vein**, and **valves of the coronary sinus**. Incorporation of the sinus venosus tissue into the dorsal wall of the right atrium results in remodeling of the right atrial chamber and the formation of the **right atrial appendage** (the **right auricle**).

The terminal crest now delimits the trabeculated right atrium from the smooth-walled sinus venarum (see Fig. 12-18*B*). The **sinoatrial node**, the cardiac pacemaker, is an important element of the cardiac conducting system and is located at the junction of the superior caval vein and the terminal crest. The cardiac impulse generated in the sinoatrial node reaches

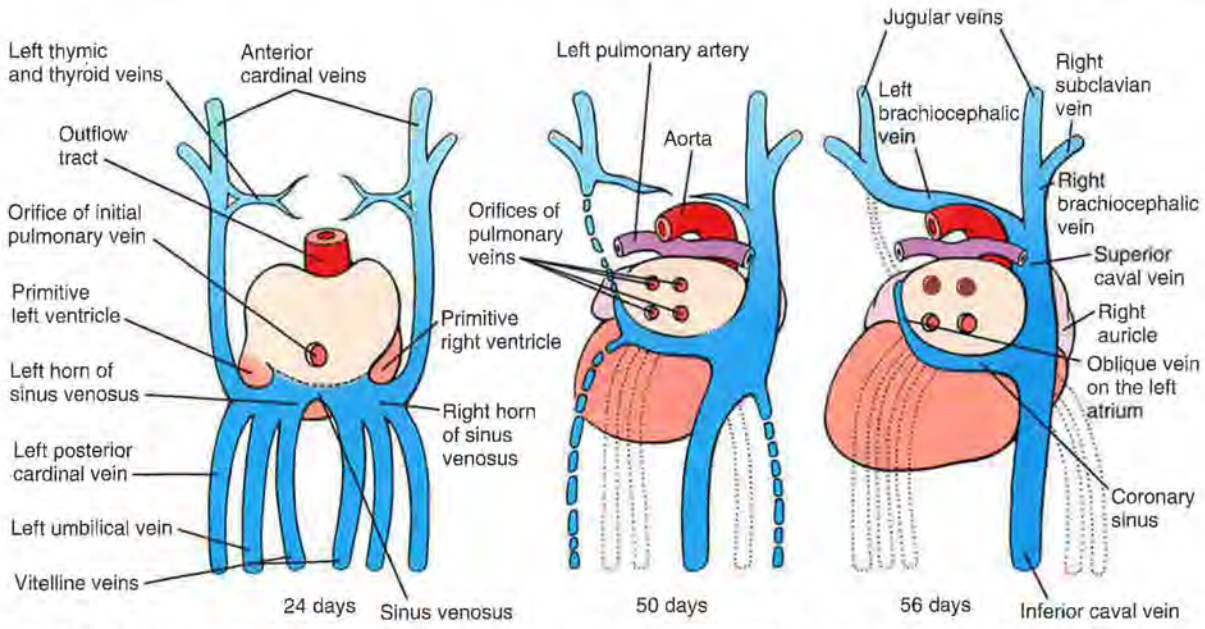


Figure 12-17. Remodeling of the inflow end of the heart between weeks four and eight so that all systemic blood flows into the future right atrium. The left sinus horn is reduced and pulled to the right side. It loses its connection with the left anterior cardinal vein and becomes the coronary sinus, draining blood only from the heart wall. The left anterior cardinal vein becomes connected to the right anterior cardinal vein through an anastomosis of thymic and thyroid veins, which form the left brachiocephalic vein. A remnant of the right vitelline vein becomes the terminal segment of the inferior caval vein (covered in Chapter 13).

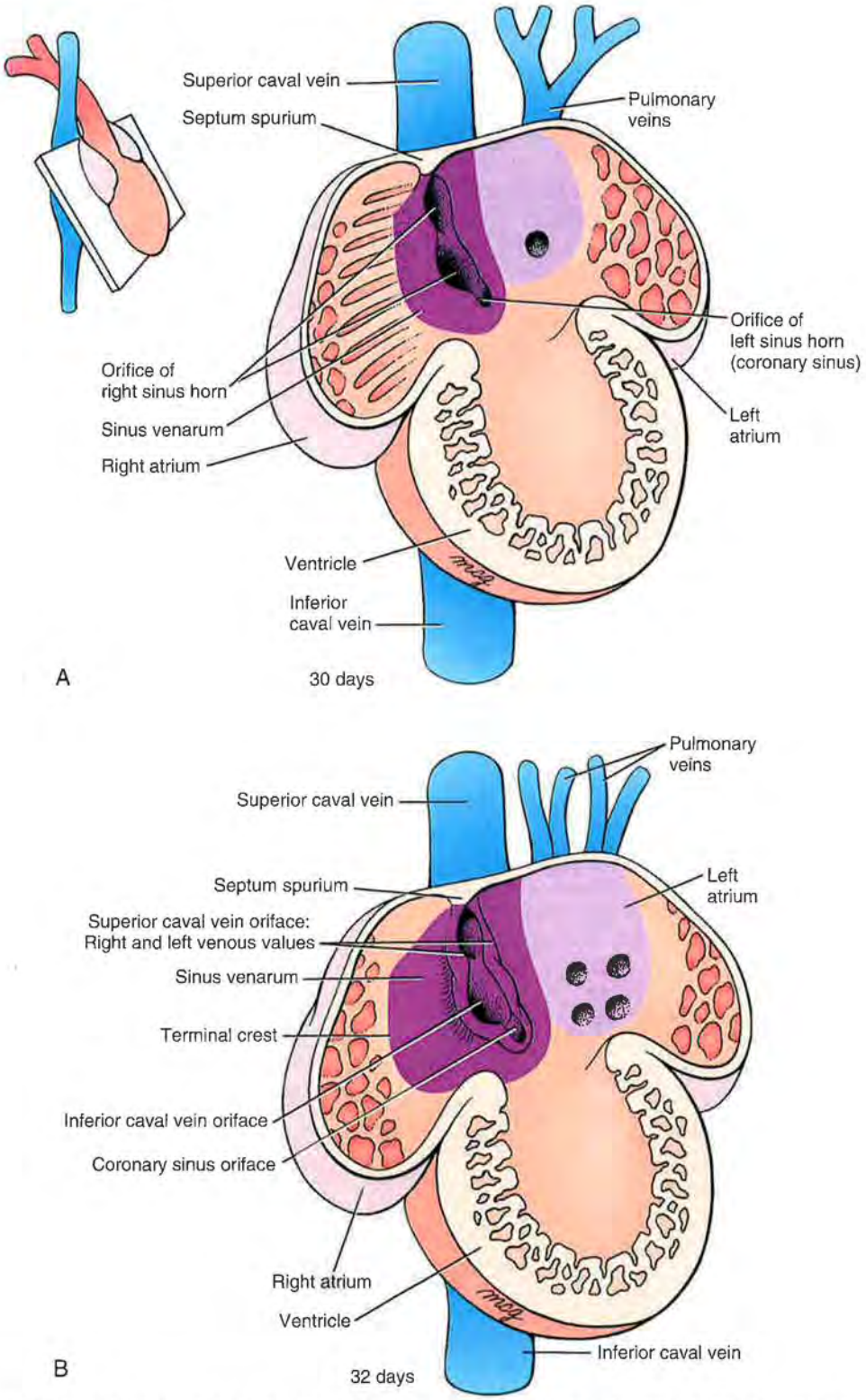


Figure 12-18. Initial differentiation of the primitive atrium. *A,* During the fifth week, the primitive atrial tissue on the left and right sides is displaced ventrally and laterally to form the trabeculated portion of the atria and auricles of the mature heart. On the right side, the right sinus horn is incorporated into the dorsal wall of the right side of the atrium as the smooth-walled sinus venarum. Meanwhile, a single pulmonary vein develops in the left side of the primitive atrium and then branches twice to produce two right and two left pulmonary veins. The sinus venarum continues to expand within the dorsal wall of the future right atrium. *B,* Further differentiation of the atrium. Later in the fifth week, the pulmonary vein system begins to undergo intussusception into the left dorsal wall of the primitive atrium. The first four pulmonary branches are incorporated into the dorsal wall of the left side of the primitive atrium, completing the formation of the smooth-walled part of the future left atrium.

Case 2:12-md-02342-CMR Document 1561-22 Filed 03/01/16 Page 21 of 23

the **atrioventricular node** using several preferred pathways.

While the right atrium is being remodeled during the fourth and fifth weeks, the left atrium undergoes a somewhat similar process. During the fourth week, the **pulmonary vein** originates as a midline structure within the caudal **dorsal mesocardium**, which connects the lung rudiments to the dorsal wall of the developing common atrium. From its initial midline position, the pulmonary vein shifts to the left (see Figs. 12-17, 12-18*A*) as a result of asymmetrical growth of a projection of second heart field mesenchymal cells called the **dorsal mesenchymal protrusion** or **spina vestibuli**. The pulmonary vein promptly splits into right and left pulmonary branches, which bifurcate again to produce a total of four pulmonary veins. These veins then grow toward the lungs, where they anastomose with veins developing within the mesoderm investing the bronchial buds (covered in Chapter 11). As a result of intussusception, the pulmonary venous system opens into the left atrium initially through a single orifice and eventually through four orifices forming the definitive pulmonary veins (see Fig. 12-18*A, B*), where they form the smooth wall of the definitive left atrium. The trabeculated left side of the primitive atrium is also displaced ventrally and to the left, where it forms a **left atrial appendage** (the **left auricle**).

SEPTATION OF HEART

Animation 12-4: Partitioning of AV Canal.
Animations are available online at StudentConsult.

Structural and functional partitioning of the heart into four chambers is accomplished through the process called **valvuloseptal morphogenesis**, which encompasses septation (formation of septal structures) and valvulogenesis (formation of valves). Major events for cardiac septation occur between days twenty-eight and thirty-seven of gestation. Two basic processes play key roles in generating septa. Differential growth and remodeling are mainly responsible for generating the muscular ventricular and atrial septa, but these processes alone never fully partition the heart chambers. For that, endocardium-derived and neural crest cell–derived cushion tissue is required. In the atrioventricular and outflow tract regions, while cardiac looping continues, extracellular matrix is secreted between endocardium and myocardium, chiefly by the myocardial layer (Fig. 12-19*A*). This essentially causes the endocardial layer to balloon into the lumen of these two regions. Near the completion of cardiac looping, some of the endocardial cells in the atrioventricular and outflow tract regions undergo an **epithelial-to-mesenchymal transformation** (EMT), generating endocardium-derived mesenchyme that invades this extracellular matrix, proliferates, and differentiates into connective tissue. These mesenchyme-filled bulges (in the atrioventricular region) and ridges (along the length of the outflow tract) are often referred to as **cushion tissues** (Figs. 12-19*B*, 12-20).

After the initial formation of the endocardium-derived atrioventricular cushions, **epicardium-derived mesenchymal cells** also populate the atrioventricular cushions. As covered later in the chapter, not only does the cushion tissue of the outflow tract contain endocardium-derived cells, but these ridges are also invaded by neural crest cells. Thus, the cushion tissue of the outflow tract consists of both mesoderm-derived mesenchymal cells **(endocardium-derived cushion tissue)** and ectoderm-derived mesenchymal cells **(neural crest cell–derived cushion tissue)** (Fig. 12-19*B*). Proper development of atrioventricular and outflow tract cushion tissues is essential for completing septation. Two major atrioventricular cushions fuse and contribute to the separation of the atria and ventricles, generate the membranous (or fibrous) portion of the ventricular and atrial septa, and, together with lateral cushions, are involved in the formation of atrioventricular valves (see Fig. 12-19*B*). The outflow tract cushions are involved in separation of the aorta from the pulmonary artery, ventricular septation, and in formation of the semilunar valves.

In the Research Lab

EPITHELIAL-TO-MESENCHYMAL TRANSFORMATION DURING ENDOCARDIAL CUSHION CELL FORMATION

The epithelial-to-mesenchymal transformation (EMT) of the endocardium can be separated into two major steps: activation (signaling) of the event, which includes induction and cell-cell separation of a subpopulation of endocardial cells; and (2) delamination and invasion of endocardium-derived cells into underlying extracellular matrix. Once populating the extracellular matrix, these cells proliferate and differentiate into various connective tissue cell types.

What triggers the EMT of the endocardium, and why does this process occur only in the atrioventricular and outflow tract regions of the heart? The answer to this fundamental question is still unclear. Early studies using chick embryos and three-dimensional tissue culture models show that only the atrioventricular and outflow tract myocardium is competent to induce EMT of the endocardium, and that only atrioventricular and outflow tract endocardium is capable of responding. The inducing factor(s) is (are) released into the extracellular matrix by the myocardium, but the precise nature of this signal is still unclear. One possibility is a multicomponent aggregate referred to as the ES (EDTA soluble) complex. Expression of this complex within the heart is restricted to the atrioventricular and outflow tract regions, and antibodies directed against this complex block EMT.

One of the earliest signs of endocardial activation is that a subset of endocardial cells hypertrophy (in this case, the rough endoplasmic reticulum enlarges and the Golgi apparatus becomes more prevalent). Soon, this is followed by morphological signs of cell-cell separation in a subset of endocardial cells and is accompanied by downregulation of cell-cell adhesion molecules, including N-Cam (neural cell adhesion molecule), VE-cadherin (vascular endothelial-cadherin), and Pe-Cam-1 (platelet endothelial cell adhesion molecule-1). If these cell-cell adhesion molecules are not downregulated, EMT fails.

Endocardial EMT recapitulates many of the same steps as the EMT responsible for gastrulation and neural crest cell formation (covered in Chapters 3 and 4, respectively). In chick embryos,

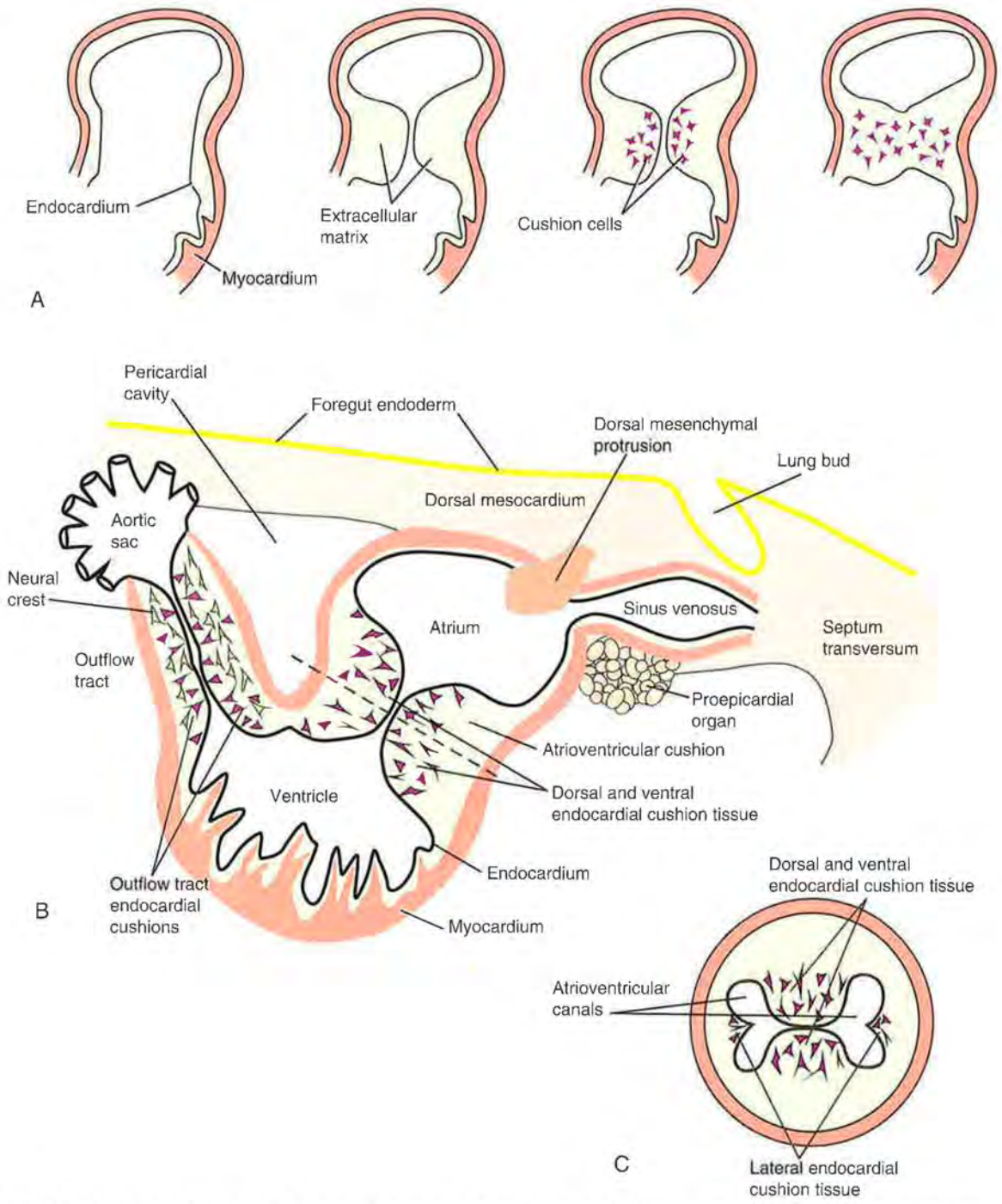


Figure 12-19. Cushion tissue formation. *A,* Steps in the formation of endocardium-derived cushion tissue. The myocardium deposits a unique extracellular matrix between the endocardium and itself at a specific stage in development. This induces an epithelial-to-mesenchymal transformation of the endocardium, resulting in the generation of migrating endocardial cushion cells necessary for cardiac septation. *B,* Sites of cushion tissue formation in the heart. Endocardium-derived cushion tissue forms in the atrioventricular region and the outflow tract region (which is also populated by invading neural crest cells). Eventual fusion of opposing cushion tissues forms the atrioventricular canals, outlets of both ventricles, the aorta and pulmonary trunk, and membranous portions of the interatrial and interventricular septa. Dashed line represents the level of the cross section illustrated in *C* and shows the atrioventricular cushion tissue and canals and the small lateral cushion tissue.

Case 2:12-md-02342-CMR Document 1561-22 Filed 03/01/16 Page 23 of 23

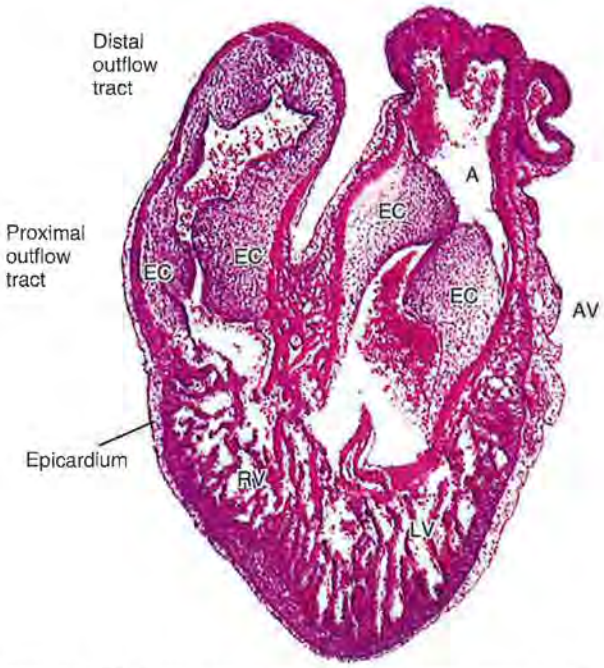


Figure 12-20. Photomicrograph of a sagittal section through a chick embryo heart showing endocardial cushion (EC) tissue surrounding the atrioventricular canal and within the outflow tract. A, Atrium; AV, atrioventricular region; RV and LV, right and left ventricles, respectively.

expression of snail2 (a zinc finger transcription factor) is upregulated in endocardial cells before EMT and during early migration of cushion cells, and blocking Snail2 expression prevents EMT of the endocardium in tissue culture. Notch1 and several of its ligands (covered in Chapter 5) are expressed in mouse endocardium at the time of EMT initiation, and notch1 null mice exhibit impaired EMT and develop hypoplastic endocardial cushion tissue. This impaired EMT correlates with reduced expression of snail and failure to downregulate VE-cadherin. Once activated, endocardial cells begin to extend filopodia into the extracellular matrix and to upregulate invasive cell markers (e.g., matrix metalloproteinases, serine proteases, hyaluronate synthetases, rho-associated kinases). This is soon followed by the transformation of this endocardial subset into mesenchymal cells that migrate and invade the extracellular matrix between endocardium and myocardium.

There are several growth factors, growth factor receptors, and transcription factors whose expression is required for the initial phase of EMT. Members of the Tgfβ family have important roles in initiating endocardial EMT. Blocking Tgfβ2 expression or neutralizing its activity using antibodies in the chick embryo inhibits both cell-cell separation and the invasive steps leading to EMT, whereas blocking Tgfβ3 inhibits EMT only after the cell-cell separation step has occurred. An important role for Tgfβs in EMT is supported by mouse Tgfβ knockout mutants, which exhibit atrioventricular valve defects, semilunar valve defects, and atrial septal defects. At least five different Bmps (another member of the Tgfβ family) are also expressed by atrioventricular and outflow tract myocardium. In mice, Bmp2 and Bmp4 are expressed in the myocardium beneath the atrioventricular and outflow tract endocardium. Using the chick tissue culture model, knocking down Bmp2 expression significantly reduces endocardial cushion cell migration; in mouse atrioventricular endocardial cultures, Bmp2 can substitute for the myocardium. These studies show that Bmp2 expressed by the myocardium has both autocrine and paracrine effects, upregulating the expression of Tgfβ2 in both myocardium and endocardium, resulting in induction of endocardial EMT.

Both the growth factor, Vegf, and the transcription factor family, Nfatc (nuclear factor of activated T cells isoform c), play important roles in endocardial EMT and subsequent valve development. Vegf signaling activity is dose dependent and is dynamically controlled within a narrow spatial and developmental window during cushion and valve development. In mice at the onset of EMT, Vegf expression occurs in both endocardium and myocardium. If Vegf signaling is too high or too low, EMT does not occur. Specific isoforms of Nfatc (c2, c3, and c4) expressed in the myocardium are responsible for reducing Vegf signaling to levels necessary for initiating EMT. Once initiated, myocardial expression of Vegf begins to increase, and this increase is thought to play a role in terminating endocardial EMT. Both Vegf signaling and activity of the Nfatc1 isoform within the endocardium are also required for subsequent valve remodeling. Hence, proper spatial/temporal Vegf signaling and Nfatc transcriptional activity are required for EMT and valve differentiation and, if atypical, lead to abnormal heart development.

A myriad of other growth factors and growth factor receptors have been implicated or shown to have important roles in signaling endocardial EMT, including Egfs, Fgfs, and ephrins. Several other transcription factors are also important for proper cushion formation and valve development, many of which have been implicated in EMTs and mesenchymal tissue development elsewhere in the embryo, including Msx1 and Msx2, Prx1 and Prx2, Id, and Sox4.

Effects of Hyperglycemia and Hypoxia on Cushion Tissue Formation

Neonates born to diabetic mothers have an almost three-fold increased risk of having congenital heart defects. Because the risk can be reduced by strict maternal glycemic control, hyperglycemia seems to be the teratogenic agent. In mice, hyperglycemic conditions inhibit the EMT required for cushion development. Hyperglycemia inhibits the release of Vegf from the myocardium, leading to retention of Pe-Cam-1 in endocardial cells. As mentioned earlier, endocardial cushion tissue formation requires proper levels of Vegf signaling and turnover of cell-cell adhesion molecules before the EMT. The effects of hyperglycemia on endocardial EMT in mice are mimicked by blocking the bioavailability of endogenous Vegf, and this is reversed by adding back appropriate levels of Vegf. Hypoxia increases the release of Vegf and, likewise, inhibits endocardial cushion formation. Thus, the negative effects of hyperglycemia and hypoxia on endocardial EMT are likely due to failure in maintaining proper Vegf signaling.

SEPTATION OF ATRIA AND DIVISION OF ATRIOVENTRICULAR CANAL

Animation 12-5: Partitioning of Atrium.
Animations are available online at StudentConsult.

A required step in separation of the systemic and pulmonary circulations consists of partial separation of the definitive atria and division of the common atrioventricular canal into right and left canals. The mature atrial septum is formed by the fusion of two embryonic partial muscular septa: the **septum primum** and the **septum secundum**. Both of these septa have openings that allow right-to-left shunting of blood throughout gestation. This shunting is required for normal development and expansion of the left atrium and left ventricle, and it permits oxygenated blood from the umbilicus to bypass the developing pulmonary system and enter the systemic circulation.